CCXVIII. THE SYNTHESIS OF RESERVE CARBOHYDRATE BY YEAST.

I. SYNTHESIS FROM GLUCOSE AND MALTOSE AND THE INFLUENCE OF PHOSPHATE THEREON.

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In the course of an investigation into the carbohydrate and fat metabolism of yeast, Smedley-MacLean and Hoffert [1923] showed that a mild ale yeast stored significantly larger quantities of carbohydrate when it was incubated in maltose solution than when incubated in solutions of glucose, fructose or sucrose of similar concentration. They concluded that maltose was directly assimilated by the yeast and built up into reserve carbohydrate and pointed out that this conclusion was in agreement with the generally accepted view that the maltose unit was present in the glycogen molecule [*cf.* Irvine, 1923]. Their data also indicated that the addition of alkali phosphates to the non-oxygenated carbohydrate solutions in which the yeast was incubated raised the total amount of carbohydrate stored by the yeast cell [1924].

We have now undertaken an analysis of the reserve carbohydrate stored by the yeast cell under different conditions with particular reference to the glycogen content.

The methods employed by various investigators for separating the carbohydrates of yeast are based either on Salkowski's method [1894] of extraction of the yeast with 2% KOH or on Pflüger's method for the estimation of liver glycogen in which 60% KOH solution is used for the extraction. By using dilute KOH, glycogen and yeast gum were obtained in solution, whilst with repeated use of strong KOH, these together with a further fraction of carbohydrate were dissolved. The latter according to Salkowski [1914] consists of the so-called erythrocellulose obtained by him in 1894 by autoclaving with water the residue after dilute KOH extraction. This substance was identical in properties with glycogen, gave only glucose on hydrolysis but had a lower specific rotation than glycogen, $[\alpha]_D$ 173·7°. Meigen and Spreng [1908] claimed that, after further purification, Salkowski's product had $[\alpha]_D$ 112°, and obtained a substance with the same rotation by extracting with 15% KOH the residue left after extracting the original yeast with 0·25% KOH. Daoud and Ling [1931], however, autoclaved with 2% KOH the residue after extraction with 2% KOH at normal pressure, thus obtaining only glycogen and yeast gum together with inorganic phosphate and silica. This latter method is stated to bring into solution some 50% more of this residue than did Salkowski's method of autoclaving with water. This fraction therefore probably contained a large part of the carbohydrate that Salkowski failed to dissolve, which gave mannose and glucose on hydrolysis and was named by him "achroocellulose".

It seems therefore that a portion of the carbohydrate of yeast is present as glycogen and yeast gum in soluble form whilst another portion is rendered soluble by the action of KOH, then appearing as glycogen and gum and possibly also some substance closely resembling glycogen. Whether the insolubility of the latter fraction is due to its condition in the cell, possibly to the polymerisation of the soluble molecules or to esterification with phosphoric and silicic acids as suggested by Daoud and Ling [1931], is not established, nor has the least soluble fraction been fully examined for other polysaccharides.

In our experiments, by one treatment of the yeast with 60% KOH, we obtained glycogen and yeast gum in solution and an insoluble solid residue. This latter, which though insoluble in water gave a red colour when treated directly with iodine, must correspond with the achroocellulose and erythrocellulose described by Salkowski and also with the esterified glycogen and yeast gum of Ling. We have referred to this fraction as yeast insoluble carbohydrate, thus making no assumptions as to its nature.

Method of experiment.

The liquid yeast was filtered under diminished pressure and, after washing, 7 g. of the yeast were taken for analysis, of which 1 g. was used for the estimation of total carbohydrate, 1 g. for the determination of dry weight and 5 g. for the separation of the individual carbohydrates. Similar portions of 7 g. weight were added to the various media which were to be investigated. After incubation at 25° for the given period, generally 2 days, the yeast was filtered off, washed and the total amount from each medium weighed: one-seventh of the total was taken for estimation of the dry weight, one-seventh for determination of total carbohydrate and five-sevenths for separation of the individual carbohydrates. In some experiments, at the end of 2 days, a sample of the medium was withdrawn, the residual sugar estimated and the appropriate sugar added to bring up the sugar content to the original strength, the incubation being continued for another 48 hours. In other experiments, this process was repeated at the end of 4 days, so that the total time of incubation reached 6 days.

The solutions compared were 5% glucose and 5% maltose with and without the addition of 0·05% PO_4. The phosphate was added by introducing a suitable amount of a solution containing 1·4 g. KH_2PO_4 and 4·9 g. Na_2HPO_4, $12H_2O$ per 100 ml. It is important that the specimens of phosphate should be tested to see that they are free from any traces of fluoride.

Total carbohydrate was determined by boiling the yeast for 2 hours with *N* HCl solution and estimating the reducing carbohydrate in the filtrate by Bertrand's method. This method was used throughout for estimating reducing sugar, the result being calculated in all cases as glucose.

Glycogen was estimated by digesting the yeast with 60% KOH for 3 hours on the water-bath, diluting and making up to 70% alcohol as described by Mayer [1923]. After standing, the liquid was decanted and the precipitate filtered off, washed with alcohol and ether and digested with hot water, the washing being continued until the filtrate gave no red-brown colour with iodine. A solution was thus obtained, containing the glycogen and yeast gum, and a residue of insoluble carbohydrate. An aliquot part of the solution was used for the estimation of glycogen and gum: the rest was saturated with ammonium sulphate, thus precipitating the glycogen, which was filtered off, hydrolysed and estimated as reducing sugar in the hydrolysate. Insoluble carbohydrate in the residue was hydrolysed and similarly estimated. In all cases the carbohydrate was

Table I.

Period of incubation days	Original yeast				After incubation in glucose solution				After incubation in glucose + phosphate solution				After incubation in maltose solution				After incubation in maltose + phosphate solution			
	Total carbohydrate	Glycogen	Gum	Insoluble carbohydrate	Total carbohydrate	Glycogen	Gum	Insoluble carbohydrate	Total carbohydrate	Glycogen	Gum	Insoluble carbohydrate	Total carbohydrate	Glycogen	Gum	Insoluble carbohydrate	Total carbohydrate	Glycogen	Gum	Insoluble carbohydrate
	g. glucose per 10 g. yeast				g. glucose per 10 g. yeast				g. glucose per 10 g. yeast				g. glucose per 10 g. yeast				g. glucose per 10 g. yeast			
2	0·67	0·16	—	—	0·85	0·29	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	0·67	0·32	0·18	—	0·87	0·36	0·23	—	—	—	—	—	—	—	—	—	—	—	—	—
2	0·60	0·18	0·12	—	0·77	0·12	0·12	—	—	—	—	—	—	—	—	—	—	—	—	—
2	0·55	0·12	0·13	—	0·77	0·18	0·09	—	—	—	—	—	—	—	—	—	—	—	—	—
2	0·52	0·24	0·16	0·23	1·15	0·51	0·23	0·36	—	—	—	—	1·47	0·79	0·35	0·30	1·82	0·67	0·49	0·47
2	0·52	0·21	0·11	0·21	1·07	0·57	0·18	0·34	1·52	0·79	0·36	0·40	1·15	0·67	0·24	0·22	1·35	0·76	0·33	0·27
2	0·50	0·37	0·20	0·15	0·75	0·25	0·17	0·32	—	—	—	—	1·37	0·72	0·28	0·42	1·77	0·86	0·41	0·50
2	0·67	0·40	0·14	0·17	0·95	0·50	0·19	0·23	1·27	0·78	0·14	0·27	—	—	—	—	1·75	1·04	0·36	0·21
4	—	—	—	—	0·80	0·50	0·16	0·26	1·45	0·70	0·28	0·43	1·42	0·81	0·27	0·49	1·52	0·83	0·33	0·55
4	—	—	—	—	0·87	0·64	0·21	0·17	1·40	0·99	0·27	0·25	1·42	1·01	0·43	0·11	1·47	0·93	—	0·13
2	—	—	—	—	0·72	0·33	0·18	0·19	1·02	0·50	0·28	0·37	—	—	—	—	—	—	—	—
2	0·70	—	—	0·15	—	—	—	—	1·35	—	—	0·57	—	—	—	—	—	—	—	—
2	—	—	—	—	0·95	0·59	—	—	1·37	0·74	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	0·25	—	—	—	0·69	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	0·82	—	—	—	1·04	—	—
2	—	—	—	—	—	—	—	—	—	0·45	—	—	—	—	—	—	—	0·90	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	0·79	—	—	—	1·02	—	—
2	—	—	—	—	—	—	—	—	—	0·54	—	—	—	—	—	—	—	1·32	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·75	—	—
Means	0·59	0·24	0·14	0·18	0·84	0·39	0·17	0·27	1·34	0·62	0·27	0·45	1·37	0·78	0·31	0·31	1·61	1·03	0·38	0·35

hydrolysed by heating for 2 hours on the water-bath with *N* HCl, this method having been compared with the more usual one for the hydrolysis of glycogen with 2·2 % HCl for 3 hours and found to give the same results.

Results (Table I).

Total carbohydrate. The amount of carbohydrate stored was increased after incubation in the glucose solution but after incubation in maltose or in a glucose-phosphate medium this increase was approximately doubled. Addition of phosphate to the maltose medium caused a further increase of the total carbohydrate stored.

Glycogen. The glycogen showed more consistent variations with the differences in media than did the storage of the other carbohydrates. Glucose produced a definite increase in glycogen content compared with that of the original sample of yeast: where the same sample of yeast was used, incubation in glucose-phosphate medium always produced more glycogen than incubation in a pure glucose solution. The glycogen content of the yeast from the maltose medium was always very high; it far exceeded that from the glucose medium and generally, but not quite invariably, that from the glucose-phosphate solution.

Considerable variations were found in the power of the different samples of yeast used to form glycogen, although the yeast was obtained from the same brewery. Thus for several weeks samples would give relatively high storage followed by a period in which in all experiments relatively low storage was obtained. It is therefore important that corresponding experiments should be carried out with the same sample of yeast in the different media.

In the majority of the experiments where samples of yeast incubated in maltose and maltose-phosphate media were compared the glycogen content of the latter was the higher: the difference was however usually not so marked as that between the glycogen values of the yeast incubated respectively in glucose and in glucose-phosphate solutions, and in some cases the amount of glycogen from a maltose-phosphate medium was less than from a maltose medium.

Insoluble yeast carbohydrate (so-called cellulose). The content of insoluble carbohydrate was raised after incubation in either glucose or maltose solution. The maltose was perhaps slightly more efficient, but no such marked difference as occurs in the case of glycogen storage was observed. The addition of phosphate produced a definite increase in the amount of insoluble carbohydrate stored, the effect being somewhat greater when the phosphate was added to the glucose than to the maltose solution. On the whole the glucose-phosphate medium appeared to be the most favourable for the development of this fraction.

Yeast gum. This was estimated by subtracting the weight of glycogen from the total soluble carbohydrate present. In some cases it was confirmed by estimation of the gum by precipitating with Fehling's solution and hydrolysing. Here an increase appeared to have been obtained after incubation in all the carbohydrate media investigated except glucose; maltose and glucose-phosphate media were equally favourable. The maltose-phosphate medium was on the whole the best but the difference was not sufficiently striking to be regarded as significant. When the gum was estimated directly the differences in storage appeared to be even less marked.

Summary.

1. The addition of phosphate to glucose or maltose media caused increased storage of glycogen, yeast gum and yeast insoluble carbohydrate by the yeast cell.

2. The glycogen stored by yeast incubated in a maltose medium far exceeded that stored when the yeast was incubated in a glucose medium and usually exceeded that stored in a glucose solution of like concentration to which phosphate had been added.

3. This appears to be the first recorded instance of a direct biological synthesis of glycogen from maltose.

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